An Abstract of Results of Laboratory Examinations of Patients with Yusho and of Animal Experiments

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An extensive clinical study of Yusho and many animal experiments were carried out by the Yusho study group in Kyushu University in order to clarify the mechanism of toxic actions of Kanechlor. Most of the findings have been published in Japanese with brief English abstracts. I hope this abstract will help in the understanding of the important observations obtained by these studies.

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Table 1. Laboratory findings of patients with Yusho.

Item	Laboratory findings	Reference
Blood (I)		
1. Red blood cells	Decreased in most severe cases.	3
2. Leucocytes	Increased in most severe cases.	1
3. Blood platelets	Normal	3
4. Coagulation time	Normal	3
5. Prothrombin time	Normal	3
6. Blood sedimentation rate	Normal except for a few cases	3
7. Hemoglobin	Decreased in most severe cases	1, 3
Blood (II) serum lipids		
1. Total lipid	Increased	3
	725.0±169.5 (mg %) 9 cases of grade IV*	
2. Triglycerides	Increased	1, 3, 4, 8
	180.6±88.2 (mg %) 9 cases of grade IV*	3
	69–617 (mg %) 24 cases	4
	More than 300 (mg %) 9 out of 24 cases	4
	189±64 (mg %) 32 adult patients	8
	Fifteen percent of the patients showed unusually high levels (more than 300 mg %) of triglycerides	20

age	No. cases	TG>300 No. case	%
0–9	52	18	34.6
10–19	89	17	19.1
20–29	68	5	7.4
30–39	85	10	11.8
40–49	58	6	10.3
50–59	28	2	7.2
60–	16	2	12.5
Total	396	60	15.1

Table 1—Continued.

Item	Laboratory findings	Reference
3. Cholesterol	Normal	1, 8
	181±43 (mg %) 27 adult patients	
4. Phospholipids	Normal	8
	8.1±1.8 (mg %) 27 adult patients	
5. Serum lipoprotein	β/α-liproprotein ratio increased.	8
	Woman with Yusho, aged 24: 2.0	
	Woman with Yusho, aged 60: 4.4	
	Man with Yusho, aged 35: 3.5	
6. Fatty acid composition of serum lipids	Analysis of cholesterol esters, triglycerides, free fatty acids, and phospholipids showed no abnormal components, except that increase of oleic acid and decrease of palmitic acid were seen in serum-free fatty acids.	4
7. Effect of glucose administration on serum free fatty acids	Levels of glucose and free fatty acids in blood were determined 30, 60, 90, 120, 150, and 180 minutes after oral administration of 50 g glucose.	8
	Two types of variation of levels were noted.	
	Type A: Blood glucose increased and later decreased. Fatty acids hardly changed.	
	Type B: Blood glucose increased and did not return to normal even after 180 minutes after glucose administration. Fatty acids decreased markedly after administration.	
	Palmitic acid decreased by glucose administration more markedly in patients than in normal persons.	
8. Post-heparin plasma lipoprotein lipase activity (PHLA)	PHLA values (FFA μEq/min/ml) 10 minutes after heparin injection in 10 normal women aged 18 to 20 in years were 0.201±0.058. Four of 5 female patients showed PHLA 10 minute values lower than 0.143, namely the mean—1 S.D. value for control, while 2 of 8 male patients had similar low values.	20
9. Lecithin-cholesterol acyltransferase (LCA)	Five patients with Yusho were examined. Mean value was 41.7% for patients which was significantly lower than control ($p=0.02$)	20
Blood (III) protein and others		
1. Total serum protein	Normal in most cases	
	6.87±0.73 (g %) 9 cases of grade IV*	3
	7.3±0.6 (g %) 35 adult patients	8
	7.3±0.5 (g %) 8 Yusho children	8
2. Serum albumin	Normal	
	56.4±5.47 (%) 9 cases of grade IV*	3
	59.4±4.2 (%) 33 adult patients	8
	58.1±6.6 (%) 8 Yusho children	8
3. Serum globulin	Increase in α_2-globulin in severe cases.	
	No significant change in other fractions.	3
	α_2-globulin	
	12.7±2.74 (%) 9 cases of grade IV*	3
	10.3±2.1 (%) 33 adult patients	8
	13.1±3.0 (%) 8 Yusho children	8
	Increase in α_1-globulin	8
	6.2±1.1 (%) 33 adult patients	
	6.0±1.0 (%) 8 Yusho children	
4. Non-protein nitrogen compounds	Normal	8
Blood (IV) electrolytes and metals		
1. Na, K, Ca	Normal	3
	Na and Ca were in normal range but K is slightly decreased.	8
	4.0±0.4 (mEq/1) 53 adult patients	

Table 1—Continued.

Item	Laboratory findings	Reference
	4.2±0.5 (mEq/1) 17 Yusho children	
2. Cl	Normal	3, 8
3. Fe	Tended to decrease.	3
	66.0±30.9 (μg %) 6 cases of grade IV*	
	Decreased	8
	90±34 (μg %) 50 adult patients	
	83±26 (μg %) 17 Yusho children	
4. Cu	Increased in severe cases	3
	183.9±61.0 (μg %) 9 cases of grade IV*	3
5. Zn	Normal	3
Blood (V) enzymes		
1. Alkaline phosphatase, serum	Increased slightly in severe cases.	1, 3, 8
	10.3±3.5 (King-Armstrong units) 65 adult patients	8
	17.4±4.9 (King-Armstrong units) 11 Yusho children	8
	14.9±9.26 (King-Armstrong units) 9 cases of grade IV*	3
2. Lactate dehydrogenase (LDH), serum	Normal in most cases.	3, 8
	278±65 (Karmen units) 24 adult patients	8
	284±24 (Karmen units) 5 Yusho children	8
3. Transaminases		
GOT	Normal	3, 8
	24±19 (Karman units) 70 adult patients	8
	36±23 (Karman units) 12 Yusho children	8
	23.0±7.7 9 cases of grade IV*	3
	28.0±10.2 cases of grade IV*	1
	24.7±8.8 cases of grade III	1
	20.0±7.3 cases of grade II	1
GPT	Normal in most cases	3, 8
	22.6±11.9 7 cases of grade IV*	3
	23±16 (Karman units) 48 adult patients	8
	21±11 (Karman units) 10 Yusho children	8
	Mean 30.1 cases of grade IV*	1
	Mean 24.1 cases of grade III	1
	Mean 16.0 cases of grade II	1
Blood (VI) others		
1. Bromsulfalein (BSP) test	Delayed in several adult cases.	3
	5.9±5.19 (%) 8 cases of grade IV*	
2. Icterus index	Normal	3
	3.3±0.66 8 cases of grade IV*	
3. I^{131} resin sponge uptake (Triosorb test)	Normal	3
Urine		
1. Protein	Negative	3
2. Glucose	Negative	3
3. Urobilinogen	Negative in most cases	3
4. Urinary neutral 17-ketosteroids	Elevation of ratio of etiocholanolone/androsterone was noted, indicating adrenocortical hyperfunction but urinary excretion of total 17-ketosteroids was in normal range.	8
	Etiochol./androst.	
	2 Normal women aged 28: 0.6, 1.0	
	2 Normal men aged 20 and 28: 0.4, 0.8	8
	2 Yusho women aged 35: 1.7, 2.0	
	2 Yusho men aged 37 and 39: 0.9, 2.6	

Table 1—Continued.

Item	Laboratory findings	Reference
	Total 17-ketosteroids 23 adult Yusho women: 5.7±1.8 (mg/day) 13 adult Yusho men: 7.3±2.8 (mg/day)	8
	Urinary total 17-ketosteroids (17-KS) and 17-hydroxycorticosteroid (17-OHCS) were determined in 50 male and 45 female patients. Positive correlation was found between 17-KS and 17-OHCS. 42% of patients exceeded normal range in both steroids in males and females.	19
	Analysis of major components of urinary 17-KS was made in 9 male (19 to 57 years in age) and 7 female (16 to 51 years in age) patients. For androsterone, etiocholanolone, and dehydroepiandrosterone, female patients seemed to be lower than control, while male patients seemed to be higher.	

Sex	17-Ks	Normal (mg/day)	Patients (mg/day)
M	Pregnanediol	0.70±0.17	0.84±0.46
	Androsterone	2.95±1.02	3.28±1.19
	Etiocholanolone	1.83±0.55	2.64±0.84
	Dehydroepiandrosterone	1.55±0.75	1.96±1.18
	Total	6.4±1.8	7.9±3.2
	Determined by Zimmermann reaction	10.6±2.3	11.6±3.2
F	Pregnanediol	0.95±0.55	1.34±0.91
	Androsterone	2.02±0.97	1.32±0.60
	Etiocholanolone	1.35±0.48	1.03±0.40
	Dehydroepiandrosterone	1.01±0.40	0.93±0.48
	Total	4.4±1.7	3.8±1.0
	Determined by Zimmermann reaction	6.1±2.4	7.2±1.9

5. Amino acids

Two patients were examined. A remarkable increase in excretion was noted for taurine (5,641; 8,103 μM/day), β-aminoisobutyric acid (247; 1,042 μM/day), glycine (1,865; 2,997 μM/day), and histidine (743; 1,170 μM/day). Increase was also noted for threonine, serine, and Tyrosine. — 8

Fatty acid analysis of acne and skin fat

1. Cheese-like material collected from acne eruptions and fat in the sweat of patients — 1

	cheese-like material %	Fat in sweat	
		Face %	Body %
myristic	2.7	11.7	9.1
palmitic	21.5	41.2	41.0
palmitoleic	10.4	23.5	22.7
stearic	16.3	11.8	9.1
oleic	30.4	11.8	18.1
linoleic	18.3		
linolenic	0.4		

Table 1—Continued.

Item	Laboratory findings	Reference
2. Yusho acne and non-Yusho acne	Yusho acne contained more stearic and oleic acids than did non-Yusho acne. Linoleic acid was present only in the former. The former contained 10 times more cholesterol than the latter.	8

	Cheese like materials		
	from ordinary acnes of many persons %	from acnes of members of a Yusho family	
		1 %	2 %
myristic	11.6	3.0	8.3
palmitic	41.2	22.1	23.4
palmitoleic	17.0	10.7	6.1
stearic	13.7	16.6	31.2
oleic	16.5	31.5	23.5
linoleic	none	16.1	7.5

Biopsy		
1. Liver	Reduction of rough endoplasmic reticulum and hypertrophy of smooth endoplasmic reticulum. Mitochondria showed morphological heterogeneity. Many filamentous inclusions were present in the matrix. Giant mitochondria were frequently encountered.	5, 22
2. Skin	Twenty-four biopsy specimens from 18 patients were examined. Marked hyperkeratosis and cystic dilation of hair follicles; marked increase of melanine in basal cells of epidermis.	9
Neurological findings	Slowing of sensory nerve conduction velocities of radial and sural nerves was noted in 9 of 23 patients examined. Motor nerve conduction velocities of ulnar and tibial nerves were shown to be unaffected except for 2 patients in whom they decreased.	6
Otorhinolaryngological findings	No audiometric abnormality and no disturbed vestibular function.	7
Ocular signs	Hypersecretion of meibomian gland; abnormal pigmentation of conjunctiva; no particular lesion in intraocular tissues. Increase of melanine granules was noted electronmicroscopically in cytoplasm of epithelial cells, especially in basal cells of conjunctiva. Innumerable electron dense particles, 300–400 Å in diameter, were in the cytoplasm of basal cells.	

* Grade IV means clinically most severe cases.

Table 2. Findings of animal experiments

Experimental	Results	Reference
1. 2 ml of olive oil containing 1% Kanechlor 400 orally given to rabbits daily for 10 days	Increase of serum triglycerides and slight increase of total cholesterol	4 (Uzawa et al.)
2. Oral administration of Kanechlor 400 to female mice, cynomolgus monkeys, and	Enlargement of liver; slight fatty metamorphosis of liver; swelling of sebaceous gland. Increase of smooth endoplasmic reticulum and decrease of rough endoplasmic reticulum in	10 (Nishizumi et al.)

Table 2—Continued.

Experimental	Results	Reference
squirrel-monkeys for a month or longer.	liver.	
3. Oral administration of 0.1 g of Kanechlor per Kg per day to rats for 4 weeks.	Loss of body weight, hepatomegaly, and marked increase in serum lipid components were noted. Serum triglycerides reached a level as high as 10 times the control level. Serum cholinesterase activity was not affected.	11 (Tanaka et al.)
4. Oral administration of the rice oil used by a Yusho patient to hairless mice.	Hyperkeratosis, cystic dilatation of hair follicle and sweat-gland, marked hepatomegaly, fatty degeneration of liver.	12 (Inagami et al.)
5. Distribution and elimination of components of Kanechlor 400 orally administered in a single dose of 2.0 mg/body to female mice.	Each component of Kanechlor 400 was equally absorbed from gastro-intestinal tract. Concentration of Kanechlor 400 in skin one day after administration was twice that in liver and kidney. Kanechlor 400 was retained in skin more than in other tissues. Tetrachlorobiphenyls were almost completely eliminated from tissues in three to four weeks, but penta- and hexa-chlcrobiphenyls were retained 9 to 10 weeks after administration.	13 (Yoshimura et al.)
6. Distribution and excretion of ^{3}H-Kanechlor orally administered in a single dose of 500 μc or 25 mg/body to Wistar King male rats weighing about 150 g.	Radioactivity in skin, adipose tissue, liver, adrenal gland and gastrointestinal tract was higher than in plasma 3 days after administration. Radioactivity was noted in some tissues after 8 weeks. Urinary excretion of Kanechlor was limited, while 70% of the dose given was in feces. A small portion of radio-activity excreted during the first day was in the phenolic fraction. More phenolic metabolites and less unchanged components were excreted in the feces on the second and third day.	14 (Yoshimura et al.)
7. Effect of oral administration of Kanechlor 400, 500, 600 and their components, 0.2 ml of a solution of 6.8 mg/0.2 ml/100 g of body weight, daily for 3 days, on liver microsomes of male rats.	Kanechlor 400, 500, 600: Hepatomegaly. Microsomal protein per gram of liver increased in proportion to chlorine content of Kanechlor. Amount of cytochrome P-450 and specific activities of O-dealkylase (p-nitrophenetol), N-demethylase (p-chloro-N-methylaniline), and aniline hydroxylase, increased, particularly with Kanechlor 500. 4,4′-dichlorobiphenyl: Slight increases seen. 3,4,2′,4′-tetrachlorobiphenyl / 3,4,3′,4′-tetrachlorobiphenyl: Increases noted, but less than with Kanechlor 400. 3,4,3′,4′-tetrachlorobiphenyl was a more potent inducer than the others. 2,4,5,3′,4′-pentachlorobiphenyl: Most marked increases were noted in cyt. P450 and specific activities of 3 enzymes. 3,4,5,3′,4′,5′-hexachlorobiphenyl: The increase was larger than that induced with the tetra chlorobiphenyls but less than that with the pentachlorobiphenyl. Phenobarbital: Resembling Kanechlor 400 in enzyme induction capability. The above microsomal enzyme activities and the microsomal components started to increase 12 hours after administration of 3,4,3′,4′-tetrachlobiphenyl, reaching the maximum after about 1 week. Thereafter, activity levels tended to decrease but did not return to normal even after 6 weeks. Microsomes, particularly their membranes, contained the largest amount of H^{3}-Kanechlor-400 administered orally, as compared with mitochondria or lysosomes.	15 (Fujita et al.)
8. Oral administration of Kanechlor 400 or 500 to female rats	Hexobarbital sleeping time was markedly shortened by pre-treatment with Kanechlor 400 or 500 in rats. Minimum effec-	16 (Komatsu et al.)

Table 2—Continued.

Experimental	Results	Reference
(Wistar King), about 100 g in weight, in order to see effects of the administration on *hexobarbital sleeping time*	tive dose was daily 5 mg and 2 mg/Kg for 3 days for Kanechlor 400 and 500, respectively. When 40 mg/Kg of Kanechlor 400 or 500 was given daily for 3 days, the effect lasted for 3 weeks by the former and for 6 weeks by the latter. Ethionine inhibited the shortening of hexobarbital sleeping time by Kanechlor. Anabolic steroids, chloroquine, glutathione, α-mercaptopropionylglycine, essential phospholipids, diphenyl, and sulfadiazine did not modify the sleeping time reduction by Kanechlor.	
9. Oral administration of daily dose of 0.5, 2.5, 5, 50 mg of Kanechlor to rats for 13 to 21 days.	Growth was inhibited by the treatments. No weight increase at daily dose of 5 mg, and a weight decrease at 50 mg. Plasma triglycerides did not increase at daily dose up to 5 mg, and slightly exceeded the control at dose of 50 mg, while plasma cholesterol and phospholipid increased definitely by increasing amount of Kanechlor.	17 (Nagai et al.)

Daily dose Kanechlor (mg)	Triglyceride μM %	Cholesterol mg %	Phospholipid mg %
Control	109±26	83±12	4.7±0.8
0.5	98+19	118+26	5.8+1.7
2.5	89+21	102+11	7.2+1.2
5.0	89+18	125+28	7.1+1.3
50.0	130+37	178+18	13.4+2.2

The administrations hardly affected concentration of triglyceride, cholesterol, phospholipid in liver except for daily administration of 50 mg Kanechlor, which caused slight increase of triglyceride and cholesterol. They slightly increased triglyceride in skin but hardly affected cholesterol and phospholipid. A definite reduction of triglyceride in perirenal adipose tissue was noted.

10. Oral administration of Fraction A and B of Kanechlor 400 to rats, each daily dose 1 mg on every fourth day for 40 days, 10 mg total. Fr. A: Boiling point 123–124°C/0.01 mmHg, 3.12 chlorine atoms per biphenyl average. Fr. B: Boiling point 140–142°C/0.01 mmHg, 3.84 chlorine atoms per biphenyl average.	Animals grew in weight with Fr. A but lost weight with Fr. B, which caused marked atrophy of abdominal adipose tissue. Lipid content of adipose tissue around kidney decreased when Fr. B was given but did not when Fr. A given. Plasma triglyceride decreased with Fr. B but did not with Fr. A.	17 (Nagai et al.)

Animal	Triglyceride μM %	Cholesterol mg %	Phospholipid mg %
Control	130±18	66±5	5.7±0.4
Fr. A	128±10	66±7	5.1±0.4
Fr. B	83±21	57±5	5.2±0.3

11. *Lipid in liver.* Oral administration of daily dose of 8 ml of olive oil containing 1% Kanechlor for 3 and 11 days to 2 female rabbits.	Total lipid and glycerides of liver increased, but concentration of serum glycerides was normal after administration for 3 consecutive days, but increased abnormally after administration for 11 consecutive days.	18 (Ito et al.)

Lipid	Control (n=1)	Poisoned for 3 days	Poisoned for 11 days

Table 2—Continued.

Experimental	Results			Reference
		(n=1)	(n=1)	
	Total lipid %/dry wt.	17	29	30
	Glycerides	7.1%	24.3%	25.3%
	Cholesterol	6.3	6.1	10.8
	CGP Lecithin	22.5	30.0	9.7
	Lysolecithin	7.4		7.6
	Sphingomyelin	2.9		4.8
	EGP	16.9		14.4
	SGP	9.0		4.5
	PI	6.8	39.6	5.1
	Glycolipid	5.2		6.7
	Others	15.9		11.1
	Serum glycerides (mg %)	52	65	1,690
12. Oral administration of daily dose 0.5 or 0.3 mg/Kg of Kanechlor 400 each to 40 adult Wistar rats.	Marked or moderately impaired motor function, decreased motor conduction velocity, early stage of segmental demyelination and loss of large nerve fibers were noted.			21

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